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Antinematodal Activity of Some Malaysian Plant Extracts against the Pine Wood Nematode, *Bursaphelenchus xylophilus*

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Abstract: Methanolic extracts of 79 Malaysian plants representing 42 families were assessed for antinematodal activity against *Bursaphelenchus xylophilus* using a fungal-feeding assay. Extracts of 27 plants from 19 families showed antinematodal activity, while 52 species were inactive. Five extracts (*Sauropus androgynus*, *Eugenia polyantha*, *Areca catechu*, *Piper betle* and *Piper nigrum*) exhibited very strong activity against *Bursaphelenchus xylophilus* at a minimum effective dose (MED) of 0·625 mg per ball. Strong antinematodal activity (MED: 1·25–2·5 mg per ball) was shown by the extracts of *Spondias cyntherea*, *Codiageum variegatum*, *Euodia glabra* and *Cicca acida*. Eleven extracts (*Carica papaya*, *Ipomoea aquatica*, *Ocimum basilicum*, *Leea gigantea*, *Pithecellobium jiringa*, *Crypteronia paniculata*, *Myristica fragrans*, *Murraya koenigii*, *Leucaena leucocephala*, *Melastoma malabathricum* and *Morinda citrifolia*) demonstrated moderate activity between MED of 5 and 10 mg per ball, and weak activity was observed in seven extracts (*Ipomoea batatas*, *Cymbopogon citratus*, *Garcinia atroviridis*, *Psophocarpus tetragonolobus*, *Tamarindus indica*, *Allium odorum* and *Stenochalaena palustris*).

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1 INTRODUCTION

Plant-parasitic nematodes (phytonematodes) consist of about 2000 species[1] from a total of about 500 000 species[2] of nematodes, which include free-living and host-associated species. This low numerical representation is, however, unreflective of the extent of world-wide economic loss, worth US$70 billion to the agricultural sector, attributed to phytonematodes.[3] Until now, the agricultural sector has relied heavily upon the use of commercial synthetic nematicides, comprising primarily carbamate, organophosphate and organohalide formulations, for phytonematode control.[4] Unfortunately, the utilisation of commercial nematicides, many of which are widely used as general pesticides, poses several problems. Some commercial synthetic nematicides exhibit high phyto-mammalian toxicity since the mode of action involves ubiquitous metabolic pathways. The

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adverse effects of some commercial synthetic nematicides on human health has led to the withdrawal of some products from the market. For example, DBCP (1,2-dibromo-3-chloropropane) was deregistered when it was found to promote both low sperm counts in human males and carcinogenesis.[5] Secondly, most commercial synthetic nematicides are xenobiotics. They may thus be recalcitrant to biodegradation, and hence exhibit high residual accumulation and leaching to cause environmental problems such as groundwater contamination. Thirdly, the high cost of commercial nematicides renders their use unfeasible especially in tropical developing countries where a limited range of nematicides are available in the market in spite of the severe phytonematode infestation. These concerns have amplified the need for discovering less toxic, environmentally acceptable and cheaper substitutes for the present commercial nematicides. Plants are considered to be an important source of antinematodal compounds capable of overcoming some of the problems associated with the present commercial nematicides. Some of these natural compounds are structurally complex which makes them inimitable by chemical synthesis. The efficacy of whole plants such as *Tagetes* species or the plant parts and extracts of *Azadirachta indica* A. Juss, as well as isolated phytochemical compounds such as asparagusic acid, for controlling phytonematodes through either or both nematistatic (inhibitory) and nematicidal (killing) action has been well documented.[6–9]

In this study, extracts of Malaysian plants comprising some traditional vegetables and medicinal species were assessed for antinematodal activity against the migratory endoparasitic pine wood nematode, *Bursaphelenchus xylophilus* (Steiner & Buhrer) Nickle. The pine wood nematode was chosen as a general model to represent plant-parasitic nematodes, as it is one of the most successful phytonematodes to be cultured *in vitro*. In order to facilitate the evaluation of a large number of plant extracts, the convenient 'cotton ball on fungal mat' method was used.[10]

2 MATERIALS AND METHODS

2.1 Plant materials

Most of the plants were collected from the Medicinal Plants Unit at Universiti Pertanian Malaysia (UPM) and identified by Dr Abdul Ghani Yunus. Some species were collected at other locations in the states of Selangor and Kelantan. Herbarium voucher specimens were prepared and deposited at the Department of Biotechnology, Universiti Pertanian Malaysia. The choice of plant parts was based on their use in folkloric medicine and as traditional vegetables.

2.2 Preparation of plant extracts

Fresh plant parts (20 g) were soaked in methanol (100 ml) for one week. The methanolic plant solutions were then evaporated at 40°C under reduced pressure and subsequently freeze-dried. The dried plant residues were redissolved in 80% (v/v) ethanol to prepare working stocks of 200 mg ml^{-1}.

2.3 Preparation of nematodes

The fungus, *Botryis cinerea* Pers., was cultured on potato dextrose agar medium in Petri dishes (diam. 90 mm) at 26°C for 10 days. Petri dishes with full-grown fungus were inoculated with *Bursaphelenchus xylophilus* and left until fungal mycelia were completely consumed. The cultured nematodes (mixed stage) were separated from the culture medium by the Baerman funnel technique and enumerated on a grid under a microscope (×20). An aqueous suspension of the nematode (*c.*15 000 nematodes ml^{-1}) was prepared by appropriate dilution for use as a working stock.

2.4 Antinematodal assay[10]

Petri dishes (diam. 35 mm) containing potato dextrose agar medium were seeded with *B. cinerea* for four days at 22°C. Cotton balls (diam. 5 mm) containing 20 mg of plant extract were placed at the centre of the fungal mat in each dish. The working stock of nematode suspension (0·1 ml) was added into each cotton ball, and the dish was kept at 26°C for 116 h. The antinematodal activity (inactive or active) was evaluated based on the ability of the nematodes to consume the fungal mat. Consumption of the fungal mycelia indicated the absence of antinematodal activity and *vice versa*. Each test extract was evaluated in triplicate. If an extract was found to be active, cotton balls were impregnated with serially diluted concentrations of the extract to determine the minimum effective dose (MED). The MED value is defined as the lowest dose of test extract to completely inhibit the nematodes from consuming the fungal mat.

3 RESULTS AND DISCUSSION

Results of the antinematodal activity of the extracts of 79 plant species from 42 families are shown in Table 1. At the preliminary screening dose of 20 mg per ball, 27 species (34%) belonging to 19 families showed antinematodal activity; 25 of the 27 species were traditional vegetables. In another study,[11] we also found a prevalence of biological activities, viz. antimicrobial and cyto-

TABLE 1
Activity of Plant Extracts Tested Against *Bursaphelenchus xylophilus*

Family and Species	[a]*Part used*	[b]*MED* (*mg per ball*)
Acanthaceae		
Asystasia intrusa Blume	LSt	−ve
Gendarussa vulgaris Nees	LSt	−ve
Amaranthaceae		
Aerva lanata Juss.	L	−ve
Amaryllidaceae		
Crinum asiaticum Linn.	L	2·5
Anacardiaceae		
[c]*Spondias cytherea* Sonn.	L	−ve
Anisophylleacea		
Anisophyllea disticha Baill.	LSt	−ve
Annonaceae		
Goniothalamus macrophyllus Hook. f.	St	−ve
Cactaceae		
Epiphyllum oxypetalum Haw.	LSt	−ve
Cannaceae		
Canna edulis Ker.	L	−ve
Capparidaceae		
[c]*Cleome rutidosperma* DC.	L	−ve
Caricaceae		
[c]*Carica papaya* Linn.	Sh	10
Connaraceae		
Santaloides floridum Kuntze	L	−ve
Convolvulaceae		
[c]*Ipomoea aquatica* Forssk.	LSt	10
[c]*Ipomoea batatas* Lam.	L	20
Dilleniaceae		
Tetracera indica Merr.	L	−ve
Euphorbiaceae		
[c]*Cicca acida* Merr.	L	1·25
[c]*Codiaeum variegatum* Blume	L	2·5
Euphorbia antiquorum Linn.	LSt	−ve
Euphorbia tirucalli Linn.	St	−ve
[c]*Manihot esculenta* Crantz.	Sh	−ve
Ricinus communis Linn.	LSt	−ve
[c]*Sauropus androgynus* Merr.	Sh	0·625
Gesneriaceae		
Cyrtandra cupulata Ridl.	LSt	−ve
Gnetaceae		
[c]*Gnetum gnemon* Linn.	L	−ve
Gramineae		
[c]*Cymbopogon citratus* Stapf.	Rh	20
Guttiferae		
[c]*Garcinia atroviridis* Griff. ex T. anders	Fr	20
Garcinia hombroniana Pierre	L	−ve
Labiatae		
Coleus amboinicus Lour.	LSt	−ve
Coleus atropurpureus Benth.	LSt	−ve
[c]*Mentha arvensis* Linn.	L	−ve
[c]*Ocimun basilicum* Linn.	L	10
Leeaceae		
[c]*Leea gigantea* Griff.	L	10
Leguminosae		
Cassia alata Linn.	L	−ve
[c]*Cynometra cauliflora* Linn.	Sh	−ve
[c]*Leucaena leucocephala* Lam.	L	−ve
	Fr	5
[c]*Millettia atropurpurea* (Wall.) Benth.	Sh	−ve
[c]*Neptunia prostrata* Baill.	LSt	−ve
[c]*Parkia speciosa* Hassk.	Seed	−ve
[c]*Pithecellobium jiringa* Prain	Sh	10
[c]*Psophocarpus tetragonolobus* DC.	Fr	20
[c]*Tamarindus indica* Linn.	L	20

TABLE 1 *Continued*

Family and Species	[a]*Part used*	[b]*MED (mg per ball)*
Liliaceae		
[c]*Allium cepa* Linn.	L	−ve
[c]*Allium odorum* Linn.	L	20
[c]*Allium sativum* Linn.	Bulb	−ve
Cordyline fruticosa Goeppert	L	−ve
Lythraceae		
[c]*Crypteronia paniculata* Blume	Sh	10
Melastomataceae		
[c]*Melastoma malabathricum* Linn.	Fl	5
Musaceae		
[c]*Musa sapientum* Linn.	If	−ve
Myrsinaceae		
Labisia pothoina Lindl.	R	−ve
Myristicaceae		
[c]*Myristica fragrans* Linn.	Fr	10
Myrtaceae		
[c]*Eugenia polyantha* Wight	L	0·625
Melaleuca leucadendron (Linn.)	BaFrLSt	−ve
Oxalidaceae		
[c]*Averrhoa bilimbi* Linn.	LSt	−ve
Palmae		
[c]*Areca catechu* Linn.	Fr	0·625
Pandanaceae		
Freycinetia malaccensis Ridl.	St	−ve
Passifloraceae		
Passiflora foetida Linn.	Fr	−ve
Piperaceae		
[c]*Piper betle* Linn.	L	0·625
Piper nigrum Linn.	Br	0·625
	LSt	−ve
Polygonaceae		
Muehlenbeckia platyclados Meissn.	LSt	−ve
Polypodiaceae		
[c]*Stenochlaena palustris* Bedd.	Sh	20
Rubiaceae		
[c]*Morinda citriflora* Linn.	L	5
Rutaceae		
Citrus maxima Merr.	LSt	−ve
[c]*Euodia glabra* Blume	L	2·5
[c]*Murraya koenigii* Spreng	L	10
Scrophulariaceae		
Curanga fel-terrae Merr.	L	−ve
Solanaceae		
[c]*Capsicum frutescens* Linn.	Fr	−ve
[c]*Physalis minima* Linn.	Fr	−ve
[c]*Solanum torvum* Swartz	Fr	−ve
Umbelliferae		
[c]*Centella asiatica* (Linn.) Urb.	LSt	−ve
Verbenaceae		
[c]*Premna foetida* Reinw.	LSt	−ve
[c]*Vitex trifolia* Linn.	L	−ve
Zingiberaceae		
Amomum kepulaga Sprague et Burkill	LSt	−ve
[c]*Costus speciosus* Smith	St	−ve
[c]*Curcuma domestica* Valeton.	Rh	−ve
[c]*Etlingera elatior* (Jack) R. M. Smith	LSt	−ve
Hedychium longecornutum Baker	LSt	−ve
Languas conchigera Burkill	LSt	−ve
[c]*Languas galanga* Stuntz	Rh	−ve
[c]*Zingiber officinale* (Linn.) Rosc.	Rh	−ve

[a] Ba: bark; Br: berries; Fl: flower; Fr: fruit; If: inflorescence; L: leaf; Rh: rhizome; Sh: shoots; St: stem.
[b] −ve: inactive.
[c] Traditional vegetables.

toxic activities, among the traditional vegetables. The number of plants exhibiting antinematodal activity is higher than that reported earlier by Kawazu *et al.*[10] This could be attributed to the selection of plants based on ethnopharmacognostic reports and folkloric use as opposed to random selection.

Antinematodal activity was found in the families of Amaryllidaceae, Caricaceae, Convolvulaceae, Euphorbiaceae, Gramineae, Guttiferae, Labiatae, Leeaceae, Leguminosae, Liliaceae, Lythraceae, Melastomataceae, Myristicaceae, Myrtaceae, Palmae, Piperaceae, Polypodiaceae, Rubiaceae and Rutaceae. The antinematodal activity profiles of the active 27 species were classified based on the following four criteria: very strong (five species, MED: 0·625 mg per ball), strong (four species, MED: 1·25–2·5 mg per ball), moderate (11 species, MED: 5–10 mg per ball) and weak (seven species, MED: 20 mg per ball). Weak antinematodal activity was shown by the extracts of *Ipomoea batatas*, *Cymbopogon citratus*, *Garcinia atroviridis*, *Psophocarpus tetragonolobus*, *Tamarindus indica*, *Allium odorum* and *Stenochalaena palustris*. Moderate activity was exhibited by the extracts of *Carica papaya*, *Ipomoea aquatica*, *Ocimum basilicum*, *Leea gigantea*, *Pithecellobium jiringa*, *Crypteronia paniculata*, *Myristica fragrans* and *Murraya koenigii* at MED 10 mg per ball, and *Leucaena leucocephala*, *Melastoma malabathricum* and *Morinda citrifolia* at a lower MED of 5 mg per ball. Strong antinematodal activity was shown by four species, i.e. *Spondias cytherea*, *Codiageum variegatum* and *Euodia glabra* at MED 2·5 mg per ball, and only the *Cicca acida* extract at MED 1·25 mg per ball. The extracts of five species, i.e. *Sauropus androgynus*, *Eugenia polyantha*, *Areca catechu*, *Piper betle* and *Piper nigrum*, showed very strong activity at the MED of 0·625 mg per ball.

Pronounced antinematodal activity was often encountered in both the families of Euphorbiaceae and Piperaceae (Table 1). The widespread occurrence of phorbol esters and related compounds may account for the activity of euphorbiaceous plants.[10] The strong activity of piperaceous plants may be explained by the presence of antinematodal propenylphenols, piperidin- and pyrrolidin-amides.[12,13] However, only the berries of *P. nigrum* and the fruits of *L. leucocephala* showed antinematodal activity, suggesting the localised presence of antinematodal compound(s) within these plants. The antinematodal assay technique used was unable to differentiate between nematicidal and nematistatic activity. Plant extracts may exert their antinematodal activity by disrupting any of the biochemical functions associated with energy generation, neuromuscular transmission, reproduction, development and structural integrity.[14]

None of the eight species from the Zingiberaceae family showed any antinematodal activity, suggesting the absence of antinematode active compounds within this family. The remaining species that failed to exhibit antinematodal activity (arranged in order of family) includes *Asystasia intrusa*, *Gendarussa vulgaris*, *Aerva lanata*, *Spondias cytherea*, *Anisophyllea disticha*, *Goniothalamus macrophyllus*, *Epiphyllum oxypetalum*, *Canna edulis*, *Cleome rutidosperma*, *Santaloides floridum*, *Tetracera indica*, *Euphorbia antiquorum*, *Euphorbia tirucalli*, *Manihot esculenta*, *Ricinus communis*, *Cytandra cupulata*, *Gnetum gnemon*, *Garcinia hombroniana*, *Coleus amboinicus*, *Coleus atropurpureus*, *Mentha arvensis*, *Cassia alata*, *Cynometra cauliflora*, *Millettia atropurpurea*, *Neptunia prostrata*, *Parkia speciosa*, *Allium cepa*, *Allium sativum*, *Cordyline fruticosa*, *Musa sapientum*, *Labisia pothoina*, *Melaleuca leucadendron*, *Averrhoa bilimbi*, *Freycinetia malaccensis*, *Passiflora foetida*, *Muehlenbeckia platyclados*, *Citrus maxima*, *Curanga fel-terrae*, *Capsicum frutescens*, *Physalis minima*, *Solanum torvum*, *Centella asiatica*, *Premna foetida*, *Vitex trifolia*, *Amomum kepulaga*, *Costus speciosus*, *Curcuma domestica*, *Hedychium longecornutum*, *Languas conchigera*, *Languas galanga*, *Etlingera elatior* and *Zingiber officinale*.

The 27 plant species showing antinematodal activity have great potential as nematode suppressants in the form of green manure which can be incorporated with soil. Green manure is easily available in developing tropical countries owing to abundant plant resources and thus provides cheap nematicides to the farmers. Furthermore, since all the plants with antinematodal activity in this study are edible, the likelihood of mammalian toxicity is remote. However, it is possible that the antinematodal component(s) of plant extracts may become deactivated in soil. Therefore, further studies to evaluate the antinematodal activity of the extracts when applied to soil are necessary. Furthermore, the antinematodal activity of the extracts must be assessed against several phytonematode species having different parasitic habits.

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